**A Luminex-based diagnostic test reduces unneeded prostate biopsies and improves the detection of prostate cancer**

Annalisa Macagno1, Jens Goepfert2, Pierre Tennstedt3, Bruno Golding1, Thomas Steuber3, Silke Gillessen4, Thomas Joos2, Ralph Schiess1

1ProteoMediX, Schlieren, Switzerland

2NMI Tübingen, Reutlingen, Germany

3Martini-Klinik, University Hospital Hamburg-Eppendorf, Hamburg, Germany

4Cantonal Hospital St. Gallen, Oncology, St. Gallen, Switzerland

**Background and Aim:** Prostate cancer diagnosis is hampered by the high false-positive rate of PSA testing and consequently leads many unnecessary prostate biopsies. Our objective was to develop immunoassays for the biomarkers previously identified using mass spectrometry and a genetics-guided discovery approach focusing on the PI3K/PTEN cancer pathway. Here we evaluated if they are capable of distinguishing benign disease from prostate cancer.

**Methods:** Proprietary reagents including protein reference standards and antibodies were generated for the individual biomarker candidates. Subsequently, bead-based sandwich immunoassays were developed using Luminex xMAP technology and validated according to bioanalytical guidelines. The assays were then used to determine the concentration in sera collected from men before undergoing prostate biopsy at the Martini-Klinik Hamburg, Germany. In addition, total and free PSA were analyzed to calculate %fPSA using the ADVIA Centaur immunoassay system. Of the 474 men included in the retrospective study, 236 men had a negative biopsy and 238 were diagnosed with prostate cancer.

**Results:** Luminex xMAP multiplexing technology (coupling each antibody to different bead regions) proved to be highly efficient for the screening of generated monoclonal antibodies and the selection of antibody pairs for sandwich assays. Assays were technically validated through assessment of sensitivity, linear range, precision, reproducibility, and stability. All assays had inter- and intra-variability (CV) <15% and linearity on dilution of the analytes.

Serum concentrations of candidate biomarkers measured with these assay were subjected to statistical analyses. Whereas %fPSA alone discriminated among biopsy-positive and negative patients with an AUC = 0.650 (P <0.001; 95% CI = 0.600-0.699), logistic regression analysis revealed that the combination of the two proteins CTSD and THBS1 with %fPSA yielded an AUC = 0.845 (P <0.001; 95% CI = 0.810-0.880). At 90% sensitivity for prostate cancer, the specificity of the combination was 60% indicating that 141 of 236 negative biopsies could have been avoided. Independent training on half of the samples and testing on the remaining samples also resulted in a high AUC = 0.872 (*P* <0.001; 95% CI = 0.826-0.918) showing reproducibility of the method.

**Conclusions:** The method presented is significantly more accurate than %fPSA alone in determining the absence of prostate cancer. The implementation of the method in clinical practice has the potential to significantly lower the rate of unnecessary prostate biopsies which are negative for cancer by more than 50%.